The molecular dynamics of Trypanosoma brucei UDP-galactose 4'-epimerase a drug target for African sleeping sickness

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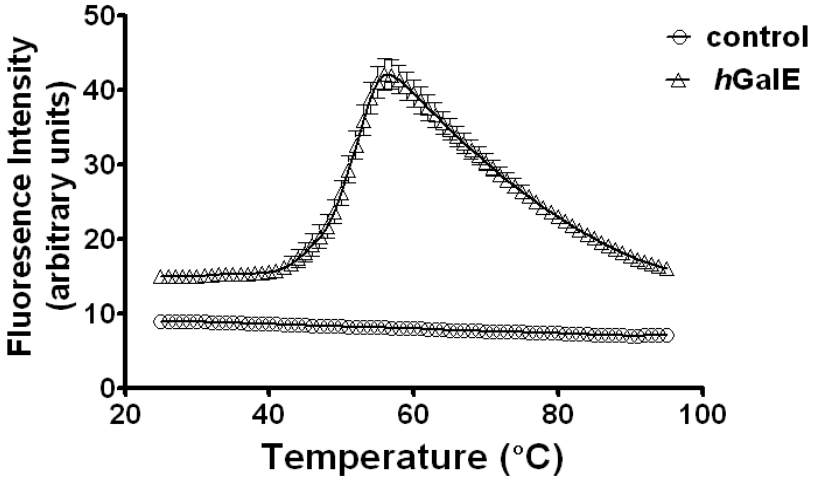
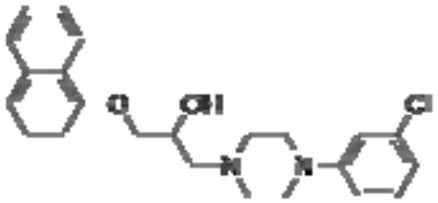
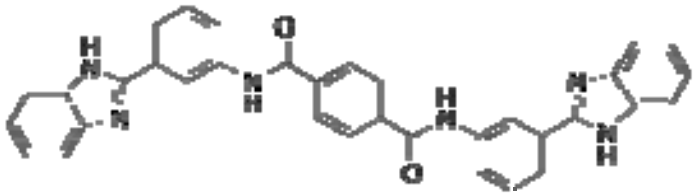
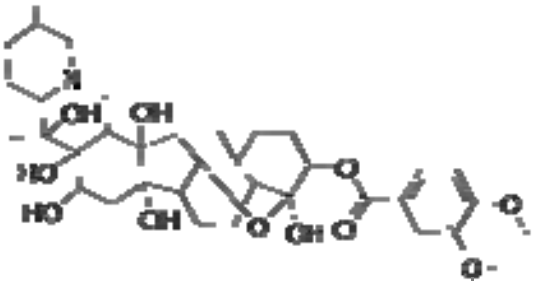
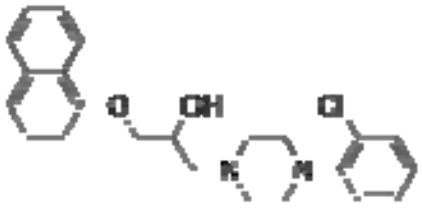
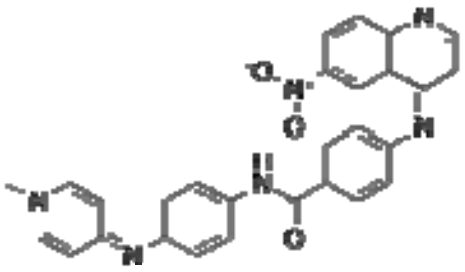
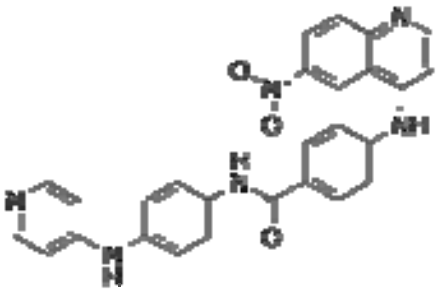
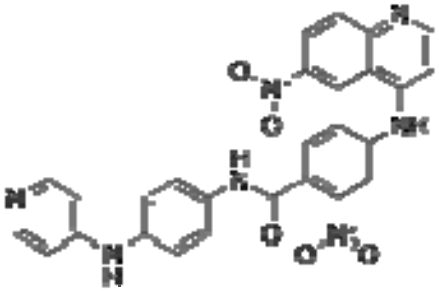


Figure S1. Thermal scanning fluorimetry of *Hs*GalE. 5 µM *Hs*GalE in 10 mM HEPES-NaOH, pH 8.8, 1% (v/v) DMSO, 5× Sypro orange showed a clear melting curve resulting in a T_m of 51.5±0.3 °C.

Table S1. *Tb*GalE Agonists

NSC ID	Structure	% inhib. @ 100 mM
91395		-167
61610		-169
7524		-191
91396		-194

260594		**-223**
146771		**-242**
202386		**-283**

Specific methods can be found in Durrant et al. (2010) *J Med Chem 53*, 5025-5032.

Table S2. Percentage activity of 20 nM *Hs*GalE in the presence of different DTP compounds

DTP Compound	% Activity
No compound	100 ± 17
91395	102 ± 32
61610	89 ± 27
7524	112 ± 3
91396	124 ± 30
260594[a]	30 ± 190
146771[a]	104 ± 57
202386[a]	41 ± 59

The reactions contained 100 μM DTP compound, 100 μM UDP-Galactose, 10 mM NAD^+, 1.2 μM HsUGDH, 10 mM HEPES-NaOH, pH 8.8, 1% (v/v) DMSO. Data are reported as the mean ± SD determined from three separate experiments. No compound resulted in a statistically significant (Student's t-test) change in activity.

[a]Compounds 260594, 146771 and 202386 gave large errors due the formation of a coloured precipitate, which prevented accurate determination of activity.

Table S3. Melting temperatures of *Hs*GalE in the presence of different DTP compounds

DTP Compound	T_m (°C)	ΔT_m (K)
No compound	51.5 ± 0.3	N/A
91395	51.3 ± 0.3	− 0.2 ± 0.6
61610	51.4 ± 0.1	− 0.1 ± 0.4
7524	51.3 ± 0.4	− 0.2 ± 0.7
91396	51.3 ± 0.3	− 0.2 ± 0.6
260594[a]	N/D	N/D
146771[a]	N/D	N/D
202386[a]	N/D	N/D

The reactions contained 5 μM *Hs*GalE, 100 μM DTP compound, 10 mM HEPES, pH 8.8, 1% (v/v) DMSO, 5× Sypro orange. The change of melting temperature, ΔT_m, due to ligand binding was calculated according to:

ΔT_m = (T_m of protein without compound) - (T_m of protein with compound)

Data are reported as mean ± SD determined from three experiments. If a compound bound to the enzyme, it would be expected to stabilize the protein's structure resulting in an increase in T_m. However, none of the compounds tested here resulted in a statistically significant (Student's t-test) change in T_m.

[a] Compounds 260594, 146771 and 202386 formed a colored precipitate, preventing determination of the melting temperature.